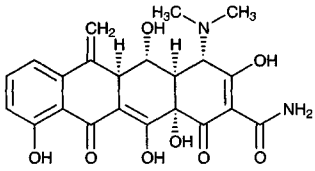
REFERENCE
Woodman,T.F.; Johnson,B.; Marwaha,R.K. Determination of methacholine chloride by ion-pair high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1982**, *5*, 1341–1348.

Methacycline



Molecular formula: $C_{22}H_{22}N_2O_8$
Molecular weight: 442.43
Merck Index: 6007
Lednicer No.: 2 227

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

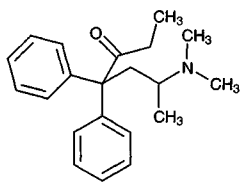
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 242.9

CHROMATOGRAM
Retention time: 11.493

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Methadone



Molecular formula: $C_{21}H_{27}NO$
Molecular weight: 309.45
CAS Registry No.: 76-99-3, 1095-90-5 (HCl)
Merck Index: 6008

SAMPLE
Matrix: blood

Sample preparation: 2 mL Plasma + 50 μL 2 μg/mL methadone in MeOH + 500 μL pH 9.6 carbonate buffer + 6 mL butyl chloride, shake on a mechanical shaker at 100 rpm for 15 min, centrifuge at 2000 g for 5 min. Remove the organic layer and add it to 3 mL 200 mM HCl. Shake for 15 min, centrifuge, remove aqueous layer. Add aqueous layer to 3 drops 60% NaOH and 6 mL butyl chloride, shake for 15 min, centrifuge. Remove organic layer and evaporate it to dryness at 50° under a stream of nitrogen, reconstitute residue in 60 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 10 μm Brownlee RP-8
Mobile phase: MeCN:MeOH:10 mM KH_2PO_4 50:30:20
Flow rate: 1
Injection volume: 40
Detector: E, Bioanalytical Systems, glassy carbon working electrode 1.20 V, Ag/AgCl reference electrode

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CHROMATOGRAM
Retention time: 16
Internal standard: methadone

OTHER SUBSTANCES
Simultaneous: oxycodone, fentanyl, meperidine, phenoperidine

KEY WORDS
plasma; methadone is IS

REFERENCE
Schneider,J.J.; Triggs,E.J.; Bourne,D.W.; Stephens,I.D.; Haviland,A.M. Determination of oxycodone in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1984**, *308*, 359–362.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 200 ng doxepin or desipramine + 100 μL 1 M NaOH + 9 mL freshly prepared hexane:isoamyl alcohol 99:1, shake vigorously for 5 min, centrifuge. Remove 8.5 mL of the organic phase and add it to 200 μL 50 mM HCl, shake well for 1 min, centrifuge, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: MeCN:0.01% phosphoric acid containing 0.01% NaCl 35:65, final pH 2.8
Flow rate: 1.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 20
Internal standard: doxepin (12.2), desipramine (14.2)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: cocaine, dextromoramide, meperidine, normeperidine, norpropoxyphene, pentazocine, propoxyphene
Simultaneous: amitriptyline, buprenorphine, chlorpromazine, codeine, desmethyldoxepin, diphenhydramine, ephedrine, imipramine, nortriptyline, oxazepam, oxycodone, pericyazine, pheniramine, propranolol, quinine, thiopropazate, thioridazine

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KEY WORDS
serum

REFERENCE

Hackett,L.P.; Dusci,L.J.; Ilett,K.F. The analysis of several nonopiate narcotic analgesics and cocaine in serum using high-performance liquid chromatography, *J.Anal.Toxicol.*, **1987**, *11*, 269–271.

SAMPLE

Matrix: blood
Sample preparation: 900 μL Plasma + 100 μL 10 μg/mL difenoxin in mobile phase, add to a C18 Sep Pak SPE cartridge at 1 mL/min, wash with 4 mL MeCN:buffer 10:90 at 1 mL/min, elute with 4 mL MeCN:buffer 40:60 at 5 mL/min, inject a 100 μL aliquot of the eluate. (Buffer was 0.08% diethylamine in water adjusted to pH 2.3 with orthophosphoric acid.)

HPLC VARIABLES

Guard column: C18
Column: 150 × 4.6 30 μm Ultracarb ODS (Phenomenex)
Mobile phase: MeCN:buffer 25:75 (Buffer was 0.08% diethylamine in water adjusted to pH 2.3 with orthophosphoric acid.)
Column temperature: 28
Flow rate: 1.5
Injection volume: 100
Detector: UV 210

CHROMATOGRAM

Retention time: 18
Internal standard: difenoxin (32)
Limit of detection: 0.25 ng

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; SPE; rat

REFERENCE

Pierce,T.L.; Murray,A.G.W.; Hope,W. Determination of methadone and its metabolites by high performance liquid chromatography following solid-phase extraction in rat plasma, *J.Chromatogr.Sci.*, **1992**, *30*, 443–447.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 1 mL 160 nM dextropropoxyphene in 1 M sodium carbonate + 6 mL n-hexane, shake horizontally for 15 min, centrifuge at 1300 g for 5 min, freeze in dry ice/acetone for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 10-80 μL aliquot.

HPLC VARIABLES

Guard column: reversed-phase (Chrompack)
Column: 100 × 3 5 μm Spherisorb CN + 100 × 4 Chiralcel-AGP (in series)
Mobile phase: MeCN:10 mM pH 5.0 sodium phosphate buffer:dimethyloctylamine 10:90:0.05
Flow rate: 0.9
Injection volume: 10-80
Detector: UV 200

CHROMATOGRAM

Retention time: 18.5 (R-(-)), 21.1 (S-(+))
Internal standard: dextropropoxyphene (15.2)
Limit of quantitation: 1.7 ng/mL

OTHER SUBSTANCES

Noninterfering: benzodiazepines, carbamazepine, ketobemidone, morphine, piroxicam, tenoxicam, valproic acid

KEY WORDS
serum; chiral; pharmacokinetics

REFERENCE
Kristensen,K.; Angelo,H.R.; Blemmer,T. Enantioselective high-performance liquid chromatographic method for the determination of methadone in serum using an AGP and a CN column as chiral and analytical column, respectively, *J.Chromatogr.A*, **1994**, *666*, 283–287.

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM
Retention time: 5.4
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: acetazolamide, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methaqualone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propranolol, protriptyline, quinidine, trazodone, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: amitriptyline, nordiazepam, norfluoxetine, propoxyphene, temazepam

KEY WORDS
plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE
Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 261

CHROMATOGRAM

Retention time: 7.53
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL 100 mM pH 6.0 phosphate buffer, do not allow to dry. 1 mL Blood + 6 mL 100 mM pH 6.0 phosphate buffer, vortex, sonicate, centrifuge, add the supernatant to the SPE cartridge, wash with water, wash with 1 mM pH 3.3 acetic acid, dry by suction, wash with 2 mL acetone: chloroform 50:50, elute with 3 mL ethyl acetate:ammonia 98:2. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pack C18
Mobile phase: MeOH:50 mM ammonium acetate 75:25 (Mix column effluent with 50 mM ammonium acetate pumped at 0.5 mL/min.)
Flow rate: 0.6
Injection volume: 10
Detector: MS, Finnigan MAT TSQ 700 tandem quadrupole, MAT TSP-2 interface, thermospray, selective reaction monitoring m/z 310-265, collision offset -10 V, repeller 100 V, vaporizer 130°, source 200°, filament on 200 μA, argon 2.5 mTorr, multiplier 1500 V, dynode 15 kV, scan time 1.20 s, MSMSC factor 10

CHROMATOGRAM

Retention time: 7.45
Limit of detection: 50 pg

OTHER SUBSTANCES

Extracted: benperidol, dextromoramide, droperidol, haloperidol, penfluridol, pimozide, pipamperidone, propoxyphene (dextropropoxyphene)

KEY WORDS

SPE; LC/MS

REFERENCE

Verweij,A.M.; Hordijk,M.L.; Lipman,P.J. Quantitative liquid chromatographic thermospray-tandem mass spectrometric analysis of some analgesics and tranquilizers of the methadone, butyrophenone, or diphenylbutylpiperidine groups in whole blood, *J.Anal.Toxicol.*, **1995**, *19*, 65–68.

SAMPLE

Matrix: blood, CSF
Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM

Retention time: 8.10
Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim

KEY WORDS
plasma; serum; column-switching

REFERENCE
Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE
Matrix: blood, tissue
Sample preparation: 2 mL Blood or 250 mg liver (homogenized with 3 parts water) or 500 mg brain (homogenized with 3 parts water) + 2 μg SKF-525-A + 1.5 mL pH 9.5 ammonium carbonate/ammonium hydroxide buffer + 10 mL hexane:isopropanol 99:1, rotate at 10 rpm for 10 min, centrifuge at 3500 rpm for 10 min. Remove the organic layer and add it to 2.5 mL 0.25 M sulfuric acid, rotate for 5 min, centrifuge at 1500 rpm for 5 min. Remove the aqueous layer and add concentrated ammonium hydroxide to make the pH 9.5, add 1.5 mL chloroform, vortex for 15 s, centrifuge at 1500 rpm for 5 min. Remove the organic layer and add 1 drop of 1% HCl in MeOH, evaporate to dryness at 50° under vacuum, reconstitute with 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 2.1 Whatman C18 pellicular
Column: 250 × 4.6 Spherisorb S-5-ODS
Mobile phase: MeCN:MeOH:buffer 48:4:48 (Buffer was 1980 mL water + 20 mL 85% phosphoric acid + 3.7 mL methanesulfonic acid adjusted to pH 3.0 with 5 M NaOH.)
Column temperature: 60
Flow rate: 2
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: 9.8
Internal standard: SKF-525-A (11.2)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: norpropoxyphene, propoxyphene, diazepam, N-desmethyldiazepam

KEY WORDS
liver; brain

REFERENCE
Rio,J.; Hodnett,N.; Bidanset,J.H. The determination of propoxyphene, norpropoxyphene, and methadone in postmortem blood and tissues by high-performance liquid chromatography, *J.Anal.Toxicol.*, **1987**, *11*, 222–224.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18

Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 11.16
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: brompheniramine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Mix 100 μL serum with 100 μL. 10 μg/mL estazolam in EtOH. Add 300μL 10% anhydrous sodium carbonate in water, 4 mL hexane, and 500 μL 2-propanol. Vortex for 2 min. Centrifuge at 1500 g for 10 min and evaporate the upper organic layer to dryness under a stream of nitrogen at room temperature. Add 100 μL mobile phase, vortex for 30 s, centrifuge at 3000 g or filter (0.22 μm). Inject a 50 μL aliquot. Urine. Mix 100 μL urine with 50 μL. 10 μg/mL estazolam in EtOH. Add 300μL 10% anhydrous sodium carbonate in water and 4 mL hexane. Vortex for 2 min. Centrifuge at 1500 g for 10 min and evaporate the upper organic layer to dryness under a stream of nitrogen at room temperature. Add 100 μL mobile phase, vortex for 30 s. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3.2 5 μm Cyclobond I-200 RSP
Column: 250 × 4.6 5 μm Cyclobond I-200 RSP
Mobile phase: MeCN:buffer:water 19:8:73 (Prepare buffer by adding 1 mL triethylamine to 70 mL water, adjusting the pH to 4.5 with glacial acetic acid, and making up to 100 mL with water.)
Flow rate: 0.4
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 15.5 (R-(-)), 17 (S-(+))
Internal standard: estazolam (22)
Limit of detection: 1 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: amphetamine, benzoylecgonine, caffeine, clonazepam, cocaine, codeine, dextropropoxyphene, diamorphine, diazepam, dionine, lorazepam, morphine, monoacetylmorphine, nalorphine, narcotine, nitrazepam, oxazepam, theophylline

Noninterfering: aspirin, barbiturates, clomipramine, imipramine, phenytoin, salicylic acid, valproic acid

KEY WORDS
serum; chiral

REFERENCE
Pham-Huy,C.; Chikhi-Chorfi,N.; Galons,H.; Sadeg,N.; Laqueille,X.; Aymard,N.; Massicot,F.; Warner,J.-M.; Claude,J.-R. Enantioselective high-performance liquid chromatography determination of methadone enantiomers and its major metabolite in human biological fluids using a new derivatized cyclodextrin-bonded phase, *J.Chromatogr.B*, **1997**, *700*, 155–163.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.753

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject 75-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco
Mobile phase: EtOH:MeCN:t-butylamine 98:2:0.05 (Prepared from 1 gal EtOH + 77 mL MeCN + 1.9 mL t-butylamine.)
Flow rate: 2
Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM
Retention time: 2.9
Internal standard: promazine (5.2)

OTHER SUBSTANCES

Simultaneous: N-acetylprocainamide, amitriptyline, amoxapine, amphetamine, buprion, chlordiazepoxide, chlorpheniramine, chlorpromazine, cocaine, codeine, demoxepam, desipramine, desmethylchlordiazepoxide, desmethyldisopyramide, desmethyldoxepin, dextropropoxyphene, diazepam, disopyramide, doxepin, hydroxyamoxapine (7- and 8-), 2-hydroxydesipramine, 2-hydroxyimipramine, 10-hydroxynortriptyline, iminostilbene, imipramine, iprindole, maprotiline, meperidine, mianserin, morphine, nortriptyline, norzimeldine, oxapam, oxaprotiline, perphenazine, procainamide, prochlorperazine, prolixin, promethazine, propoxyphene, protriptyline, pyrilamine, quinidine, thioridazine, trifluoperazine, trimeprazine, trimipramine, zimeldine
Noninterfering: thiopropazine
Interfering: chlorimipramine, fluphenazine, loxepin, phentermine, triflupromazine

KEY WORDS

normal phase

REFERENCE

Beierle,F.A.; Hubbard,R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, *5*, 279–292.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 2.68

OTHER SUBSTANCES

Simultaneous: codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, thebaine, norlevorphanol, benzylmorphine, ethylmorphine, morphine-N-oxide

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.9

OTHER SUBSTANCES
Simultaneous: metabolites
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: Pelliguard LC-CN (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 58:14:28
Flow rate: 1.2
Injection volume: 40

Detector: UV 254

CHROMATOGRAM
Retention time: 11.0
Internal standard: N-propionylprocainamide (6)

OTHER SUBSTANCES
Simultaneous: amitriptyline, atropine, butalbital, chlorpromazine, desipramine, desmethylmaprotiline, doxepin, imipramine, maprotiline, norpropoxyphene, nortriptyline, phenylpropanolamine, procainamide, prochlorperazine, promethazine, propranolol, protriptyline, quinidine, trifluoperazine, trimeprazine, trimipramine
Noninterfering: acetaminophen, allopurinol, amikacin, amoxapine, amytal, bretylium, caffeine, carbamazepine, carisoprodol, chloramphenicol, chlordiazepoxide, chlorpropamide, clonazepam, codeine, diazepam, disopyramide, droperidol, ethinamate, ethinamate, ethosuximide, fluphenazine, flurazepam, furosemide, gentamicin, haloperidol, hydrochlorothiazide, hydroxyzine, ibuprofen, kanamycin, lidocaine, loxapine, meperidine, mephobarbital, meprobamate, methaqualone, methotrexate, morphine, nafcillin, naloxone, neomycin, perphenazine, phenacetin, phenobarbital, phenytoin, prazepam, primidone, procaine, propoxyphene, reserpine, salicylamide, salicylic acid, secobarbital, spironolactone, theophylline, thiopental, thioridazine, tobramycin, valproic acid, verapamil

REFERENCE
Lin,W.-N.; Frade,P.D. Simultaneous quantitation of eight tricyclic antidepressants in serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1987**, *9*, 448–455.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 10 μm PRP-1 (Hamilton)
Mobile phase: Gradient. MeCN:20 mM ammonium hydroxide from 15:85 to 100:0 over 17 min
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 16

OTHER SUBSTANCES
Simultaneous: cocaine, codeine, reserpine, thebaine, yohimbine

REFERENCE
Keystone Scientific Catalog, 1993-4, p. 22.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 7.650

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE

Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 16.58 (A), 8.43 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after

0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: ambient (column A), 40 (columns B and C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 3.8

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone

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KEY WORDS

column-switching

REFERENCE

Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE

Matrix: urine

Sample preparation: Extract 1 mL urine.

HPLC VARIABLES

Guard column: Lichrospher 100 RP-18

Column: CHIRAL-AGP (Chrom-Tech)

Mobile phase: MeCN:10 mM pH 5.0 sodium phosphate buffer:dimethyloctylamine 10:90:0.05

Flow rate: 0.7

Detector: UV 200

CHROMATOGRAM

Retention time: 14.3 (R), 17.9 (S)

Internal standard: imipramine (26.9)

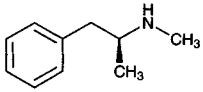
OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS
chiral

REFERENCE
Kristensen,K.; Angelo,H.R. A stereoselective HPLC method for the determination of methadone and is main metabolite in urine (using an AGP column) (Abstract 39), *Ther.Drug Monit.*, **1995**, *17*, 393–393.

Methamphetamine



Molecular formula: $C_{10}H_{15}N$
Molecular weight: 149.24
CAS Registry No.: 537-46-2
Merck Index: 6015
Lednicer No.: 1 37

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 μL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeNC:20 mM pH 6.9 phosphate buffer 10:90
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 3.79

OTHER SUBSTANCES
Extracted: ethosuximide, sulfamethoxazole, primidone

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 100 mM NaOH + 3 mL n-hexane, shake for 20 min, centrifuge for 10 min. Remove 2 mL of the organic layer and evaporate it to dryness using a vacuum centrifuge, reconstitute the residue in 500 μL 100 μg/mL (S)-(+)-benoxaprofen chloride in dried dichloromethane, let stand at room temperature for 30 min, inject a 10 μL aliquot. (Synthesis of benoxaprofen chloride is as follows. Dissolve 600 mg benoxaprofen in 50 mL toluene, slowly add 5 mL freshly-distilled thionyl chloride, reflux for 30 min, evaporate to dryness, recrystallize benoxaprofen chloride from dichloromethane.)

HPLC VARIABLES
Column: 250 × 4.6 7 μm Zorbax-Sil
Mobile phase: Cyclohexane:dichloromethane:THF 50:10:10
Flow rate: 1
Injection volume: 10
Detector: F ex 312 em 365